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View Abstract

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Commercial Relationships Disclosure (Abstract): Danielle O'Loughlin: Commercial Relationship: Code N (No Commercial Relationship) | Victoria Kearns: Commercial Relationship: Code N (No Commercial Relationship) | Hannah Levis: Commercial Relationship: Code N (No Commercial Relationship) | Carl Sheridan: Commercial Relationship: Code N (No Commercial Relationship) | Elizabeth Canty-Laird: Commercial Relationship: Code N (No Commercial Relationship)
Study Group: (none)

ABSTRACT

TITLE: Nanotopography of substrates directs the deposition of fibrillar collagen by corneal stromal cells and deposition is accelerated by macromolecular crowding

ABSTRACT BODY:
Purpose: The corneal stroma consists of roughly orthogonal lamellae composed of collagen fibrils with diameter of 25-30 nm. Traditional cell culture methods can take several months to create a thick construct when trying to replicate the stroma *in vitro*. The aims of this study were to investigate the effects of using a macromolecular crowder to accelerate collagen deposition by cells, with and without topographical cues, to efficiently recapitulate the stroma *in vitro*.

Methods: Aligned PTFE nanofibres were transferred onto glass coverslips using friction transfer. Cells isolated from human corneo-scleral rims were seeded onto coverslips, aligned versus control, and cultured in either complete media (NORM) or complete media with 75 µg/ml of carrageenan (CAR) for up to 30 days. A collagen probe was added to culture media for 90 mins before constructs were fixed with 10% neutral buffered formalin and imaged using confocal microscopy. The alignment of collagen fibres was analysed using OrientationJ, an ImageJ plugin. In addition, immunocytochemistry (ICC) was performed to analyse the deposition of collagen type I (Col I). For collagen fibre alignment, results reported are Welch's t-test, SD and n=3.

Results: Cells cultured in NORM deposited fibrillar collagen that aligned parallel to the topographical cues provided by the aligned PTFE nanofibres. At 30 days, over 70% of collagen fibres deposited by the cells on the PTFE nanofibres were aligned within ± 10°, compared to just 45% of the fibres deposited by cells on the control ($p<0.05$). ICC revealed that from as early as day 7 there was an increase in deposition of collagen type I by cells in CAR compared to NORM. When cells were cultured in CAR for a prolonged period of time, the crowded collagen appeared to aggregate and fibre alignment was not promoted. ICC analysis of the aggregates showed positive staining for Col I.

Conclusions: OrientationJ analysis shows that the cells cultured on PTFE nanofibres in NORM deposit parallel collagen fibres, emulating a lamella within the native corneal stroma. However, when cells are crowded with CAR for 30 days, the collagen deposited appears aggregated, no longer forming fibres. This suggests that whilst CAR may accelerate collagen deposition, further work is required to recapitulate the orthogonal alignment of the collagen lamellae in the native corneal stroma.

(No Image Selected)

DETAILS

PRESENTATION TYPE: Poster Only : Travel Award Applicant
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Registration Number (Abstract): (none)

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TRAVEL GRANTS and AWARDS APPLICATIONS

AWARDS: Association for Research in Vision and Ophthalmology - International (ARVO-I)|ARVO Foundation (Intl)|ARVO Members-in-Training Outstanding Poster Award|ARVO and ARVO Foundation Travel Grants|Joanne G. Angle|Women in Eye and Vision Research

AFFIRMATIONS

Affirmations: Affirmation that abstract data/conclusions have not been published; not redundant with other submissions from same investigators.

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Affirmations: Affirmation to pay Annual Meeting's full registration fee.

Affirmations: Affirmation that submission of this abstract has been approved by the Principal Investigator.

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